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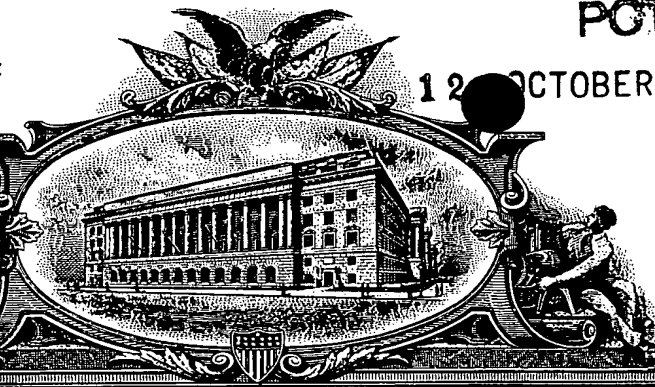
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APPLICATION NUMBER: 09/154,627

FILING DATE: September 17, 1998

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

File No. 12667-16"US" FC/ld

ASSISTANT COMMISSIONER FOR PATENTS  
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Sylvain CHEMTOB et al.

For: G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

Your petitioner prays that letters patent may be granted for the invention set forth in the enclosed specification including a disclosure, claims and declaration.

Enclosed are :

- ☒ 4 sheet(s) of drawings.
- ☒ An additional copy of this sheet and an Assignment of the Invention to HÔPITAL SAINTE-JUSTINE
- ☐ A certified copy of \_\_\_\_\_ on the basis of which the benefit of priority under 35 U.S.C. 119 is claimed.
- ☒ Declaration of small entity status.

	No. filed	Number Extra	Rate	Basic Fee \$395.00
Total Claims	7			-
Multiple Dependency Fee	-	-	-	-
Independent Claims	1			-
Base Filing Fee				\$395.00
Assignment Fee				40.00
Total				\$435.00

A cheque No. 005870 including the amount of \$435.00 to cover the Government Filing and Extra Claims Fee is enclosed.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Account No. 19-5113.

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Date: September 16, 1998

G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

BACKGROUND OF THE INVENTION

(a) Field of the Invention

5           The invention relates to development of antagonists of a G protein-coupled receptor which bind to the G protein-coupled receptor from the extracellular side.

(b) Description of Prior Art

10           Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, intestinal secretion, lipolysis, glomerular filtration, 15 mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* 46:205-229; Goetzl, 20 E.J. et al., 1995, *FASEB J.* 9:1051-10585). Prostanoids mediate their actions through binding to distinct receptors which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These receptors are coupled to heterotrimeric G- 25 proteins comprising of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. et al., 1994, *Ann. Rev. Biochem.* 63:101- 30 132).

          Of the five pharmacologically distinct prostanoid receptors for PGE<sub>2</sub>, PGI<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2 $\alpha$</sub>  and TxA<sub>2</sub> and their many isoforms, the receptor for PGF<sub>2 $\alpha$</sub> , also called FP receptor, shows limited tissue distribution, 35 predominantly expressed in corpora leutea, uterine

myometrium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is marked by tremendous rise in  $\text{PGF}_{2\alpha}$  levels and increased uterine contractility. The wide spread use of

5  $\text{PGF}_{2\alpha}$  analogues to induce labor in veterinary industry points to the primary role of  $\text{PGF}_{2\alpha}$  and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto et al., 1997, *Science* 277:81-83). In face of escalating

10 costs incurred as a result of premature births and associated complications to the neonate, such as intraventricular hemorrhage, bronchopulmonary dysplasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature

15 labor is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ . However,

20 inhibition of  $\text{PGE}_2$  is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension.

At another level,  $\text{PGF}_{2\alpha}$  has been attributed a

25 major role in dysmenorrhea, a condition which afflicts 5%-7% of premenopausal women. A pre-menstrual increase in  $\text{PGF}_{2\alpha}$  levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended

30 therapy hampered the advances in preventing premature labor and associated sequelae, and the design of such antagonists is the subject of this application.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares

35 only 47% sequence identity with  $\text{EP}_1$  receptor, and to a

lesser extent with other prostanoid receptors (Abramovitz et al., 1994, *J. Biol. Chem.* 269:2632-2636). Binding of  $\text{PGF}_{2\alpha}$  to FP receptor is followed by the activation of  $\text{G}_{\alpha\beta\gamma}$  complex, increased GTP binding  
5 by the  $\text{G}_{\alpha\gamma}$  subunit, stimulation of phospholipase  $\text{C}\beta$  activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction (Coleman, R.A. et al., 1994,  
10 *Pharmacol. Rev.* 46:205-229). The FP receptor is the only efficacious target for development of therapeutic drugs since a few  $\text{G}_\alpha$ -proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little  
15 use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors. Their efficacy will be  
20 compromised in face of tremendous increase in  $\text{PGF}_{2\alpha}$  concentrations in myometrium at the onset of labor and in menstruation. The high basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

25 It would be highly desirable to be provided with antagonists of FP receptors which do not crossreact with other prostanoid receptors and which are effective even in the presence of excess ligand.

30 SUMMARY OF THE INVENTION

One aim of the present invention is to provide antagonists of FP receptors which do not crossreact with other prostanoid receptors and which are effective even in the presence of excess ligand.

Another aim of the present invention is to provide inhibitors of FP receptors by a novel strategy to target the extracellular domains of the receptor protein.

5 In accordance with the present invention, there is provided a G protein-coupled receptor antagonist which specifically binds to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.

10 In accordance with a preferred embodiment of the present invention, the antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.

In accordance with a preferred embodiment of the present invention, the G protein-coupled receptor is the FP receptor of prostaglandin F<sub>2α</sub>.

15 In accordance with a preferred embodiment of the present invention, the antagonists of the present invention comprise amino acid sequences derived from the prostaglandin F<sub>2α</sub> receptor. Preferably, the antagonists include, without limitation, amino acid sequences of the FP receptor ILGHRDYK (PCP-8; SEQ ID NO:1) or WEDRFYLL (PCP-10; SEQ ID NO:2), protein fusions or peptidomimetic thereof.

20 In accordance with another embodiment of the present invention, the antagonists of the present invention comprise amino acid sequence derived from the second extracellular loop of prostaglandin or thromboxane receptors.

25 In accordance with another embodiment of the present invention, there is provided a method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G  
35 protein-coupled receptor antagonist or functional

derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor, thereby hampering uterine contractions.

5 In accordance with another embodiment of the present invention, there is provided a method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G  
10 protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor to hamper transduction of a signal thereby reducing the pain  
15 associated with contractions.

For the purpose of the present invention the following terms are defined below.

The expression "a G protein-coupled receptor antagonist" is intended to mean any natural or  
20 synthetic compound, peptide protein, antibody, peptidomimetic or small chemical molecules, without limitation, insofar as it can specifically bind to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.  
25 More preferably, the antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.

The expression "functional derivatives" of G protein-coupled receptor antagonist is intended to mean  
30 mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled receptor antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.

35

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 illustrates the inhibitory effects of PCP-8 and PCP-10 on FP receptor function upon stimulation with  $\text{PGF}_{2\alpha}$  in accordance with the  
5 embodiment of the present invention;

Fig. 2A illustrates the effects of PCP-8 and PCP-10 on the diameter of the microvessels of pig retina upon stimulation with either  $\text{PGF}_{2\alpha}$  or thromboxane  $\text{A}_2$  mimetic, U46619;

10 Fig. 2B illustrates the dose response of  $\text{PGF}_{2\alpha}$  on the diameter of pig microvessels treated previously with PCP-8 or PCP-10;

Fig. 2C illustrates the effects of thromboxane  $\text{A}_2$  mimetic, U46619, on the diameter of pig microvessels  
15 treated previously with PCP-8 and PCP-10;

Fig. 3A illustrates the effects of PCP-10 upon spontaneous contractions of uterine smooth muscle;

Fig. 3B illustrates the dose response of prostaglandin  $\text{F}_{2\alpha}$  in the presence/absence of PCP-8 and  
20 PCP-10 upon uterine smooth muscle contraction; and

Fig. 4 illustrates the reversal of basal tone of bovine myometrium even in the presence of FP receptor ligand,  $\text{PGF}_{2\alpha}$ .

25 **DETAILED DESCRIPTION OF THE INVENTION**

In accordance with the present invention, there is provided a new class of G protein-coupled receptor antagonists which bind to the extracellular molecular surface, thus hamper signal transduction.

30 Also provided is a novel strategy to target the extracellular loops of the receptor which contribute to the structural or functional integrity of the receptor. Antagonists thus bind to cognate elements in the extracellular surface of the receptor and prevent the



receptor function by interfering with its signal initiation or transduction.

There is provided proof of selectivity of the antagonists to FP receptor by showing an absence of  
5 their effects on a related prostaglandin receptor for thromboxane  $A_2$ , known as TP receptor which is also involved in smooth muscle contraction.

#### **Preparation of inhibitors**

##### Chemical synthesis of PCP-8 and PCP-10:

10 We have synthesized using F-moc chemistry and solid phase Merrifield method two peptides, PCP-8 and PCP-10 which are 8 amino acids in length. These peptides were purified by HPLC and their purity tested by mass spectroscopy.

15 In accordance with the present invention, a novel strategy of using peptides derived from the extracellular domains of prostaglandin  $F_{2\alpha}$  receptor, FP, to inhibit the signal transduction and the functional consequences of FP receptor. This method  
20 could be generalized to all G protein-coupled receptors. Peptides derived from the second extracellular domain of FP receptor were found to be effective inhibitors of FP receptor.

The present invention could be readily  
25 understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

#### EXAMPLE I

30 **Effects of peptides, PCP-8 and PCP-10, on ligand-induced phosphoinositide hydrolysis in mammalian cells overexpressing the FP receptor**

Both PCP-8 and -10 were tested in HEK293 cells expressing the human FP receptor. For this purpose, HEK  
35 293 cells stably expressing human FP receptor were plated in 12-well plates in DMEM medium containing 10%

fetal bovine serum, penicillin (10 U/ml) and streptomycin (10 µg/ml) and cultured in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C. After the wells were 80% confluent, the cells were labeled with  
5 2 µCi/ml of [<sup>3</sup>H]-myo inositol overnight. Next day, the cells were washed once with PBS, and incubated in 0.5 ml of Kreb's buffer containing 10 mM LiCl and indicated concentrations of PCP peptides for 30 min. PGF<sub>2α</sub> at 1µM was added to the cells and the incubation  
10 was carried out for an additional 30 min. The cells were solubilized with 0.1 N NaOH for 10 min and neutralized with 0.1 N formic acid. The lysates were collected and 1 ml each of methanol and chloroform were sequentially added and vortexed briefly. After  
15 centrifugation to separate the phases, inositol phosphates were separated by ion exchange chromatography as described below (Berridge, M.J. et al., 1983, *Biochem. J.* 212:473-482).

Briefly, the medium was discarded and the IP3  
20 synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation  
25 at 6000 x g for 10 min. The aqueous phase was applied to AG-1X-8<sup>TM</sup> (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the inositol phosphates were  
30 eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III), the eluates were counted by scintillation spectrophotometry.

The results of these experiments are shown in  
35 Fig. 1. Data are expressed as fold stimulation of

inositol phosphate synthesis by 1  $\mu$ M PGF<sub>2 $\alpha$</sub>  compared to the unstimulated controls. Both PCP-8 and -10 at 100  $\mu$ M potentially inhibited inositol phosphate synthesis initiated by the action of PGF<sub>2 $\alpha$</sub>  on FP receptor. The half maximal inhibitory concentrations for both PCP-8 and -10 were slightly less than 100  $\mu$ M.

#### EXAMPLE II

##### 10     Testing PCP peptides in porcine eyecup model of *ex vivo* vasomotricity assay

15             In order to see if the peptides could inhibit FP function using an *ex vivo* model, we chose porcine eyecup model, an *ex vivo* assay of vascular constriction in porcine retinas which we previously described and validated (Li et al., 1996 J. Pharmacol. Expt. Therapeut. 278: 370-377; Li et al., 1997 Am. J. Physiol. 273: R1283-90; Abran et al., 1997 Am. J. Physiol. 272: R995-1001). Since FP receptor densities in newborn vasculature are minimal due to down  
20 regulation by high levels of circulating prostaglandins in the perinatal period, the newborn pigs were treated with a prostaglandin synthase blocker, ibuprofen (30 mg/Kg of bodyweight/ 8 h for 24 h) to increase the density of the receptors as well as their vasomotor  
25 effects. By inhibiting circulating prostaglandins, we were able to show potent inhibition of FP receptor-mediated second messenger synthesis as well as FP-mediated vascular constriction in this eyecup model.

30             To prepare eyecups, a circular incision was made 3-4 mm posterior to ora serrata to remove the interior segment and vitreous body with minimal handling of the retina. The remaining eyecup was fixed with pins to a wax base in a 20 ml tissue bath containing 20 ml of Kreb's buffer (pH 7.35-7.45),  
35 protease inhibitors, leupetin and aprotinin (10  $\mu$ g/ml each), and equilibrated with 21% oxygen and 5% carbon

dioxide at 37°C. The preparations were allowed to stabilize for 30 min. Peptides at 100  $\mu$ M were added and incubation was continued for 30 min before the addition of PGF<sub>2 $\alpha$</sub> .

5 Cumulative concentration-responses of PGF<sub>2 $\alpha$</sub>  and TxA<sub>2</sub> mimetic, U46619, ( $10^{-10}$  to  $10^{-5}$  M) curves were constructed separately. To assess the reversibility of the antagonists, the eyecups were thoroughly washed (which would wash away the peptide) with incubation  
10 medium and concentration response curves for PGF<sub>2 $\alpha$</sub>  were determined. The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400™) and the responses were quantified by a digital image analyzer (Sigma Scan Software, Jandel  
15 Scientific, Corte Madera, CA). Vascular diameter was recorded before and 10 min following the topical application of the agent. Each measurement was repeated three times and showed <1% variability.

The results are shown in Fig. 2. The peptide  
20 PCP-10 had no effect on the basal tone (diameter of the microvessel) of the vessel (Fig. 2A; left panels). Addition of 1  $\mu$ M of PGF<sub>2 $\alpha$</sub>  potently constricted the vessel in the absence of the peptide (middle-top panel), whereas presence of PCP-10 at 100  $\mu$ M markedly  
25 inhibited PGF<sub>2 $\alpha$</sub> -mediated vasoconstriction (middle-bottom panel). The peptide had no effect on the vasoconstriction effected by 1  $\mu$ M TxA<sub>2</sub> mimetic, U46619, (right panels) acting on another prostanoid receptor coupled to constriction, namely TP receptor. Similar  
30 results were obtained for PCP-8 as well. A dose response of PGF<sub>2 $\alpha$</sub>  on the vascular diameter in the presence/absence of PCP-8 and PCP-10 peptides are presented in Fig. 2B. Both peptides abrogated the vasomotor responses even at concentrations exceeding  
35 1 $\mu$ M of PGF<sub>2 $\alpha$</sub> , suggesting, as expected, that the

peptides may be acting in a non-competitive fashion. However, the peptides had no effect on vasoconstriction produced by thromboxane A<sub>2</sub> (Fig. 2C).

5

### EXAMPLE III

#### Testing PCP peptides in porcine uterine strip of ex vivo basal contraction assay

In ex vivo experiments using porcine uterine strips, the peptides were able to prevent both basal  
10 and PGF<sub>2α</sub>-induced contraction.

Uterine tissues from non-pregnant adult pigs were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were  
15 set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as we have described (Potvin, W. et al., 1990, *Br. J. Pharmacol.* 100:341-347; Varma, D.R. and Chemtob, S., 1993, *J. Pharmacol. Expt. Ther.* 265:1096-1104). Contractions  
20 were recorded by force transducers on Grass-polygraph. Strips were incubated with or without 100 μM peptides for 30 min before adding PGF<sub>2α</sub> in step-wise increments (10<sup>-9</sup> to 10<sup>-6</sup> M). Data were expressed as percentage increase over the basal level of average tension (g).

25 A graph of spontaneous uterine contractions (known to be dependent upon prostanoids, mainly PGF<sub>2α</sub>) in the absence and the presence of 100 μM PCP-8 are shown in Fig. 3A. Addition of peptide to the strips reduced the force of basal contraction. A dose response  
30 of PGF<sub>2α</sub> on uterine contractility in the presence or absence of PCP-8 and PCP-10 peptides is shown in Fig. 3B. More than 60% (PCP-8) and 80% (PCP-10) reduction in uterine contraction was observed in all concentrations of PGF<sub>2α</sub> tested. Thus, both these  
35 peptides reduced spontaneous as well as PGF<sub>2α</sub>-induced contractions in the uterine strips.

EXAMPLE IV

**Testing PCP peptides in bovine uterine strip of ex vivo basal contraction assay**

5           Uterine tissues from non-pregnant adult bovine  
~~animals were obtained from a local slaughter house and~~  
transported to the laboratory on ice. Uterine  
myometrial strips of approximately 1 cm in length were  
set up in organ baths containing Kreb's buffer  
10   equilibrated with 21% oxygen at 37°C as described  
above. Contractions were recorded on Grass-polygraph by  
force transducers. Strips were incubated with or  
without 100  $\mu$ M peptides before adding PGF<sub>2 $\alpha$</sub>  in step-  
wise increments ( $10^{-8}$  to  $10^{-6}$  M). Data were expressed as  
15   change in basal level of average tension (g). The  
results are shown in Fig. 4. Application of PCP-10  
peptide at 100  $\mu$ M reversed the basal tone (contractile  
state) of the uterine muscle. Addition of PGF<sub>2 $\alpha$</sub>  up to  
10 $\mu$ M did not affect the relaxation produced by PCP-10  
20   suggesting that the effects of PCP peptides are  
independent of the ligand, which was also shown in the  
previous results.

While the invention has been described in  
connection with specific embodiment thereof, it will be  
25   understood that it is capable of further modifications  
and this application is intended to cover any  
variations, uses or adaptations of the invention  
following in general, the principles of the invention  
and including such departures from the present  
30   disclosure as come within the known customary practice  
within the art to which the invention pertains and as  
may be applied to the essential features hereinbefore  
set forth, and as follows in the scope of the appended  
claims.

35

SEQUENCE LISTING

<110> Hôpital Sainte-Justine  
CHEMTOB, Sylvain  
PERI, Krishna G.

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<120> G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

<130> 12667-16US FC/ld

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1 5

<210> 2

<211> 8

<212> PRT

<213> FP receptor

<400> 2

Trp Glu Asp Arg Phe Tyr Leu Leu  
1 5

WHAT IS CLAIMED IS:

1. A G protein-coupled receptor antagonist which specifically binds to the extracellular structural elements of the G protein-coupled receptor to hamper transduction of a signal.
2. The antagonist of claim 1, wherein said antagonist does not crossreact with other prostanoid receptors and is effective in the presence of excess ligand.
3. The antagonist of claim 1, wherein the receptor is prostaglandin F<sub>2α</sub> receptor (FP receptor).
4. The antagonist of claim 2, which comprises amino acid sequences of the FP receptor ILGHRDYK (PCP-8; SEQ ID NO:1) or WEDRFYLL (PCP-10; SEQ ID NO:2), protein fusions or peptidomimetic thereof.
5. The antagonist of claim 1, which comprises an amino acid sequence derived from the second extracellular loop of prostaglandin or thromboxane receptors.
6. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 5 or functional derivatives thereof.
7. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically



effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 5 or functional derivatives thereof.

### ABSTRACT OF THE INVENTION

The present invention relates to a new class of G protein-coupled receptor antagonist which specifically binds to the receptor protein structural elements, ~~thus hampering ligand-initiated signal~~ transmission and subsequent physiological effects such as smooth muscle contraction. Described herein are peptide sequences derived from the G protein-coupled receptor protein, produced by chemical methods as selective inhibitors of signal transduction associated with stimulation of the receptor by its ligand. Such peptides or molecules derived from their primary, secondary or tertiary structures may be used as effective tocolytics for the prevention of premature labor or be used for the treatment of dysmenorrhea.

# Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled G PROTEIN-COUPLED RECEPTOR ANTAGONISTS, the specification of which

☒ is attached hereto.

☐ was filed on \_\_\_\_\_ as Application No. \_\_\_\_\_  
and (if applicable) was amended on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having filing date before that of the application on which priority is claimed;

## Prior Foreign Application(s)

Number	Country	Day/Month/Year Filed	Priority Claimed
_____	_____	_____	_____

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States Application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Day/Month/Year Filed	Status (Patented, Pending, Abandoned)
_____	_____	_____

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

ROBERT MITCHELL, Registration No. 25,007; GUY HOULE, Registration No. 24, 971, PAUL MARCOUX, Registration No. 24,990, KEVIN P. MURPHY, Registration No. 26,674; ROBERT CARRIER, Registration No. 30,726; MICHEL J. SOFIA; Registration No. 37,017; FRANCE CÔTÉ, Registration No. 37,037; and JAMES ANGLEHART, Reg. No. 38,796, and address all correspondence to:

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I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Sylvain CHEMTOB	Inventor's signature <i>Sylvain Chemtob</i>	Date 16/09/98
Residence and Post Office address 6885 Banting, Montréal, Québec, Canada H4W 1G1	Citizenship Canadian	
Full name of second inventor Krishna G. PERI	Inventor's signature <i>Krishna G. Peri</i>	Date 16/09/98
Residence and Post Office address 3555 Atwater Ave., #315, Montréal, Québec, Canada H3H 1Y3	Citizenship Canadian	

Applicant or Patentee: Sylvain Chemtob et al.

Serial or Patent No.: \_\_\_\_\_ Atty. Dkt. No.: 12667-16"US" FC/ld

Filed or Issued: \_\_\_\_\_

For: G PROTEIN-COUPLED RECEPTOR ANTAGONISTS

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS  
[(37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION]**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION HÔPITAL SAINTE-JUSTINE

ADDRESS OF ORGANIZATION 3175 chemin de la Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION  
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(A) AND 501(C)(3)]  
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_)  
(CITATION OF STATUTE \_\_\_\_\_)  
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE [26 USC 501(A) AND 501 (C)(3)] IF LOCATED IN THE UNITED STATES OF AMERICA  
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_)  
(CITATION OF STATUTE \_\_\_\_\_)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9 (e) for purposes of paying reduced fees under section 41(a) or (b) of Title 35, United States Code with regard to the invention entitled G PROTEIN-COUPLED RECEPTOR ANTAGONISTS by inventor(s) Sylvain CHEMTOB and Krishna G. PERI described in

- ☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_ issued \_\_\_\_\_

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). \*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. [37 CFR 1.27]

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Richard Maheu

TITLE IN ORGANIZATION Assistant Director

ADDRESS OF PERSON SIGNING 3175 Côte Sainte-Catherine, Montréal, Québec, Canada H3T 1C5

SIGNATURE \_\_\_\_\_

DATE 16 sept '98

Treatment	Fold stimulation of IP hydrolysis
Vehicle	~20
PCP-8 (100 $\mu$ M)	~10
PCP-10 (100 $\mu$ M)	~9

Fig. 1

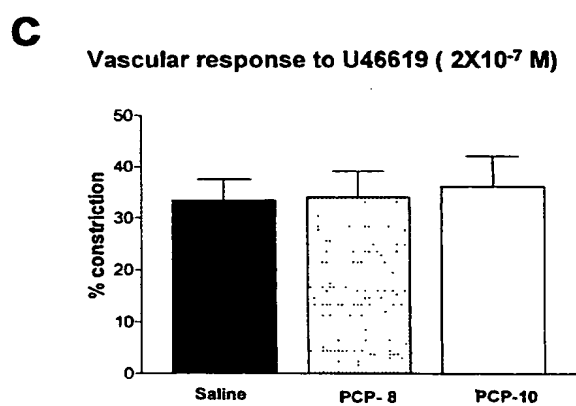
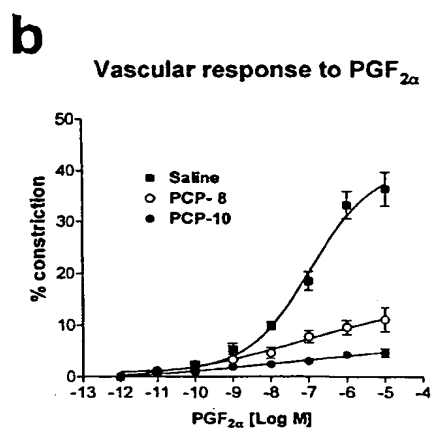
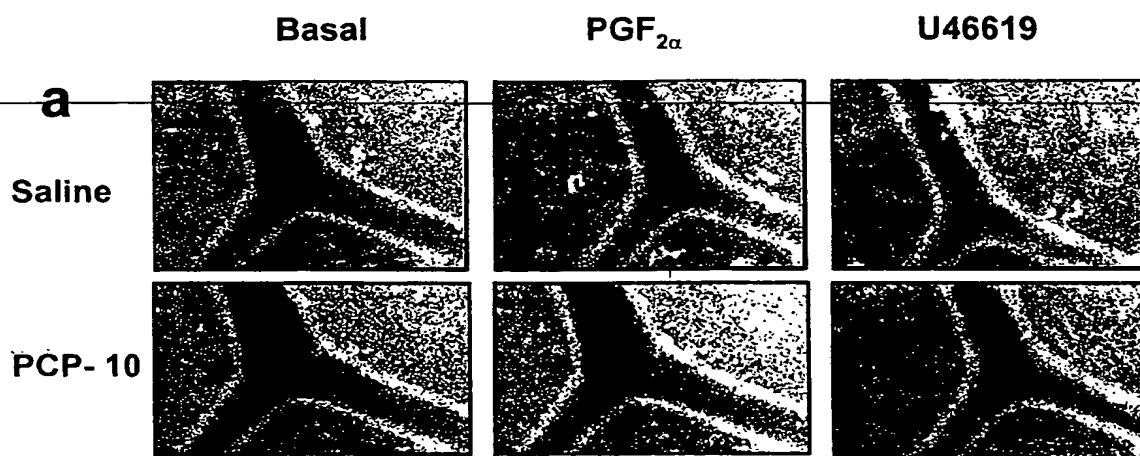
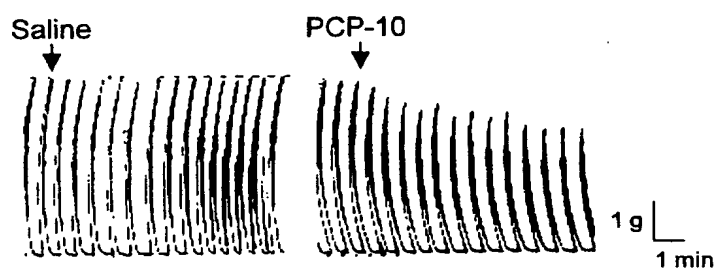


Fig. 2

a)



b)

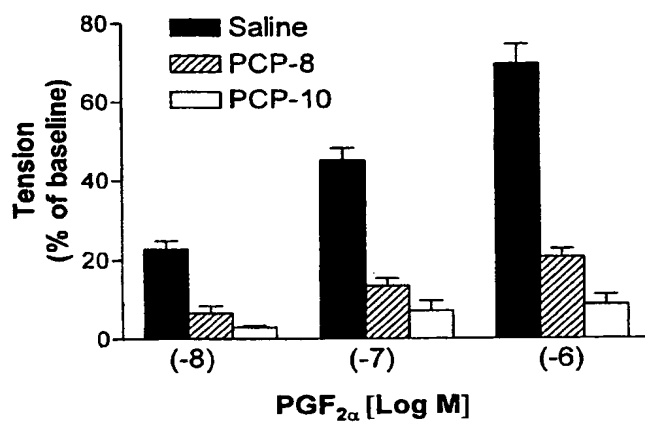


Fig. 3

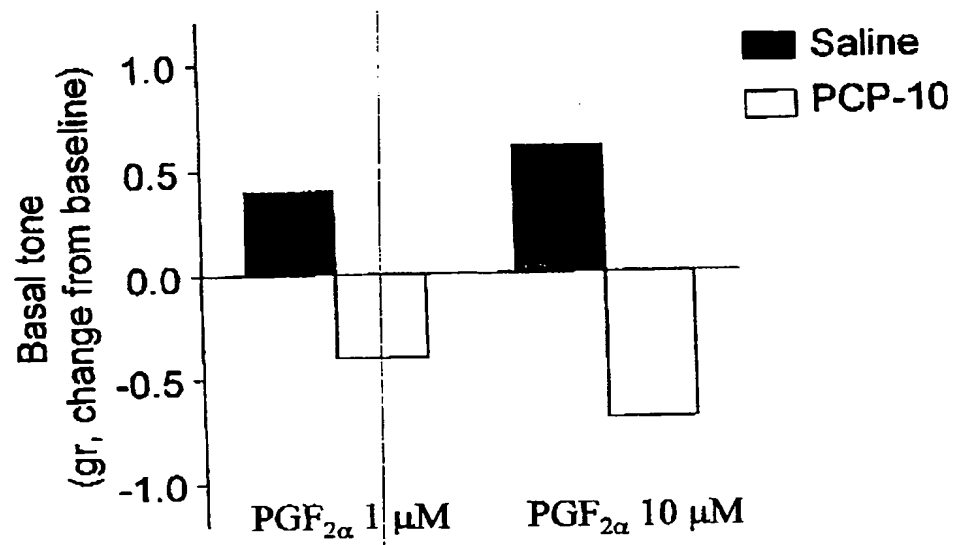


Fig. 4